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Seroprevalence and Associated Risk Factors of African Horse Sickness in Arsi and Bale Zones, Southeastern Ethiopia

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Abstract: A cross sectional study was conducted on equine from November 2010 to February 2011 to determine the seroprevalence of African Horse Sickness Virus (AHSV) antibodies and identification of potential risk factors in equine population in selected areas of Arsi and Bale zones. A total of 480 serum samples were collected. Competitive ELISA test was employed to determine the presence of African Horse Sickness (AHS) antibodies. The seroprevalence of 28.63 and 14.23% were found in the Arsi and Bale zones, respectively. The apparent seroprevalence was found to be 24.60% in donkey, 20.34% in horses and 20% in mules. The overall seroprevalence of AHS virus was found to be 21.45%. There was no significant variation between the horse, donkey and mules in the seropositivity ($p>0.05$). Statistically significant difference ($p<0.05$) in the seroprevalence was observed in the different study area, confirming the existence of agro-ecology variation in the occurrence of AHS, thus higher seroprevalence of AHS was documented in midland (31.38%) followed by highland (15.06%). Significant variation was not observed in seroprevalence among age groups and sex of equine. All age groups as well as male and female of equine population were equally affected. Knowledge base of equine owner about AHS, Clucoides vector and mode of transmission of the disease in the study areas were assessed through structured questionnaire. The survey result indicated that almost all equine owners did not know about Clucoides vector and mode of transmission. Therefore, there should be awareness reaction about AHS and Clucoides vector among the people through an organized extension package to the present study areas.

Keywords: AHS, AHSV, C-ELISA, clucoides, equine, risk factors, seroprevalence

INTRODUCTION

Ethiopia has the largest equine population, probably with highest density per square kilometer in the world and it has a total of 6.9% of the worlds and 42.4% of Africa's equine population. More over it has 65% of all African mules, almost 50% of horses and 30% of donkey (Alemayhu, 2004). Equines play an important role in the transport of farm product, fodder, firewood, agricultural inputs and construction and waste materials. Equine power is used both in rural and urban transport system which is cheap and viable. It provides the best alternative in places where the road network is insufficiently developed, or the terrain is rugged and mountainous and in cities where narrow streets prevent easy delivery of merchandise. The importance of equidaes as working animals needs to be weighed within the prevailing context of their socio economic value, their demography, distribution and the implementation of disease control program based on strategic system derived from a sound epidemiological knowledge (Wilson, 1995; Feseha, 1998). The study conducted by Feseha (1998) and Williams and Mesiga (1998), indicated that many factors contribute the poor performance of equines among which viral disease are characterized by high morbidity and mortality rate are to be mentioned.

An African horse sickness is a highly fatal insect born viral diseases of horses, mules and generally subclinical diseases in other equidae. The clinical signs and lesions result from selective increased of vascular permeability and are characterized by an impairment of the respiratory and circulatory system Radostits *et al*. (2007).

The hosts in order of decreasing severity of AHS are horses, mules, donkeys and zebras. Horses and mules have the highest mortality; donkeys have a lower mortality and African donkey have a subclinical infection. The horses are an amplifier of virus for arthropods Mellor *et al*. (1998). African horse sickness is also known as pernicious fever or typhoid fever. It is an endemic disease in equatorial, Eastern and Southern Africa from which it regularly spreads South and periodically North either along the Nile river or along the West Coast of Africa. It appears to be truly an African disease (NVI, 1974; Mellor *et al*., 1998).

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Ethiopia is facing serious and repeated outbreak of AHS in different regions. The Virus Neutralization Test (VNT) indicated that two serotypes of AHS were involved in the outbreak occurred in 2002-2003 in Southern Ethiopia (Awassa, Hossana, Wondogenet and Hagerselam), Western Ethiopia (Jimma, Bedelle, Nekemte, Horroguduru and chaliya) and Central Ethiopia (Debrzait, Meki, Zeway, Filtimo and Bekejo) serotypes 9 and 6 were isolated from blood, spleen and lymph nodes collected from 12 sick and dead animals. The identification of serotype 6 represents the first report in Ethiopia. Of the nine serotype identified, type 9 is predominantly found throughout the African content and it is the only serotype previously identified in Ethiopia Aschalew *et al*. (2005). Two research works were conducted in Wello administrative region on the pathomorphological and hematological changes in affected equidae due to AHS. The findings of the two research works indicated that AHS occurs every other years and it is very difficult to rear horses in this area. Furthermore, they have confirmed that both cardiac and pulmonary forms of the disease exist in the area (NVI, 1983).

A sero-epidemiological survey was done in selected areas of central Ethiopia and prevalence rate of 10.4, 29.7 and 10.37% from horses, donkey and mule respectively, with over all seroprevalence rate 23% was recorded by Kassa (2006). At present AHSV is endemic in tropical and subtropical areas of Africa South of the Sahara occupying a broad band stretching from Senegal in the West to Ethiopia, Somalia and Kenya in the East and extending as far as Northern part of South Africa (Mellor and Hamblin, 2004).

The study conducted by Aschalew *et al.* (2005) showed that Ethiopia has the wide spread occurrence of AHS across various ecological zone, because of large population of equines are raised in Ethiopia, the presence of multiple serotypes of such a devastating virus poses a serious economic losses. The study conducted by Keith (2005) and Kassa (2006) in Ethiopia indicate that AHS exist almost all age groups and all agro-ecological zones of the surveyed areas. Therefore, the present study is initiated to fill a gap of AHS on selected areas of Arsi and Bale Zone with the following objectives to determine the seroprevalence of AHSV in equine in different agro-ecology of Arsi and Bale Zones and to identify the potential risk factors associated with AHSV.

MATERIALS AND METHODS

Study area: A cross sectional study was conducted on equine from November 2010 to February 2011 to determine the seroprevalence of African Horse Sickness Virus (AHSV) antibodies and identification of potential risk factors in equine population in selected areas of Arsi and Bale zones. Arsi zone is found in south east of Addis Ababa, 175 km and located 6º15'-8º49'N and 38°41'-40°41'E, an altitude of 1565-3100 m above sea level (CSA, 2003). From this zone nine kebele are included in the study namely Ashebeka, Burkitu and Shalojigesa, Gendabusa, Tulujebi and Melkajebi, Gunguma, Munesa and Guridangago are selected. Bale zone is found South east of Addis Ababa 425 km at latitude and longitude of 7º7'N 40º0' E and 7.117ºN 40°E, respectively with an elevation of range 1675-2492 m above sea level (CSA, 2003). From Bale zone also nine kebele are selected for the study such as: Wajitushebe, Fasilangeso and Etitusura, Amanlema, Elanygodage, Kasowarra, Deneba, Ketaberenda and Aleltu.

The study areas are classified into two agro-ecological zones based on temperature and length of plant growing period EARO (1998). Keith (2005) adopted classification that categorizes areas with elevations of 1500 m.a.s.l or less was indicated as low land, but with elevations of 1500-2500 m.a.s.l as midland and over 2500 m.a.s.l as highland. The altitude of the study area ranges from 1565-4000 m.a.s.l. The rain fall in the area is bimodal in distribution and falls in the range of 500-1400. The minimum and maximum temperature in the study area is 4-26ºC. In the study area there are Bale Park, Batu Mountains, Wabeshebele River and Maribo River in the Bale zone where as in the Arsi Chilallo Mountains, Keter River, Welkesa River and Keleta River are found. Other water points, water reservoirs and irrigation canals were common in the study area.

Target population: The target population of the study areas was 23087 Donkeys, 45425 Horse and 3713 Mules. Donkey, horse and mules above 6 month of age, both sexes and with previous history of no vaccination against AHS were sampled. The number of mules were very low from the total sampled equidae because there was lack of access to get mule, thus it is not the representative sample of the study area.

Study design: A cross sectional study was conducted from November 2010 to the mid of February 2011 to determine the seroprevalence of AHSV by using competitive ELISA. Data on the potential risk factors associated with the occurrence of AHS were collected during sampling through recording and questionnaire administration.

Table 1: Serum sample collected from equines of different study areas

Study areas	Number of sampled			Number of equine		
Kebeles	Horse	Donkey	Mule	Horse	Donkey	Mule
Wajitushebe	20	9	-	2056	200	35
Fasilangeso	16	8	-	1863	500	15
Etitusura	20	5	-	2300	280	7
Amanlema	17	9	1	80	760	60
Elanygodage	16	8	2	681	240	40
Kasowarra	20	6	1	247	860	39
Deneba	21	7	-	2271	1041	4
Ketaberenda	20	6	-	553	280	-
Aleltu	21	6	-	438	470	-
Burkitu	21	5	-	20	470	-
Ashebeka	22	8	-	330	2806	-
Shalojigesa	23	2	-	300	100	-
Gendabusa	22	5	-	900	1603	261
Tulujebi	18	8	1	1344	1030	187
Melkajebi	10	16	-	800	920	367
Gunguma	23	7	-	750	1940	39
Munesa	21	9	-	470	600	6
Guridangago	23	5	-	890	613	32
Total	349	126	5	16293	23087	1013

Sampling method and sample size determination: Systematic random sampling was applied to select the study population; for an infinite population with 95% confidence level, 5% desired absolute precision and 50% expected prevalence, since there was no previous information on the prevalence of AHS antibodies in the study areas. The sample size was determined according to Thrusfield (2005). Even if 384 samples were the minimum sample size required, to increase the precision, 480 equine serums sample from two zones were collected.

Sampling and data collection: Whole blood of 10 mL was collected by vein puncture using sterile venoject needles and plain vacutainer tubes including needle holder under aseptic conditions. Each sample was labeled with identification number. The blood was allowed to clot over night at room temperature. The recovered serum was decanted into another tube and labeled with similar identity. The sample was transported at +4ºC then kept at -20ºC until evaluated with competitive ELISA. Area of sampling, age, sex, type of equidae (donkey, horse and mule), date of collection, agro ecology (highland or midland) and vacutainer identification number was recorded at the time of sampling. The test was done two weeks after completion of serum collection. The antigen used in the kit was VP 7 recombinant protein from the AHSV (serotype 9) and the competitive ELISA test was conducted at NAHDIC virology laboratory. The procuder of test used to identify AHSV antibodies.

Seroprevalence survey: Equine (horse, mule and donkey) serums collected from different areas are shown by Table 1.

Questionnaire survey: A questionnaire format that was aimed at assessing the potential risk factors such as management practice (stabling condition and vaccination history), presence of equine biting insects and availability of water bodies near the study areas and the knowledge base of equine owners about AHS was prepared to interview individual owners of donkeys, horse and mules. A total of 90 equine owners, 5 from each kebele were interviewed and the result was summarized.

Data management and analysis: Data recorded during sampling, laboratory findings were entered and stored in Microsoft excel spread sheet. The data were thoroughly screened for errors and properly coded before subjected to statistical analysis. The data were brought from the Microsoft excel spread sheet and analyzed using STATA 7.0 software to establish associassion (χ^2 test) between serological test results and risk factor. Tables are used to present the results and the overall positive seroprevalence are calculated by dividing total number of positive sample over the total sample and multiplying with hundred.

RESULTS

Seroprevalence survey: From the 241 serum sampled in Arsi and 239 serum sampled in Bale, the seroprevalence of AHS was found to be 28.63% (69/241) and 14.23% (39/239), respectively. Of the 480 sampled equidae from eighteen kebeles the overall seroprevalence of 21.45% was found as indicated in Table 2. The highest seroprevalence was observed in the Amanlema 51.9%, Ashebeka 46.67% and Melkajebi

Table 2: C-ELISA result of AHSV in different study areas

Zone	Kebele	Total tested	Positive	Seroprevalence %
Bale		239	31	14.23
	Wajitushebe	29	0	0
	Fasilangeso	24	1	4.170
	Etitusura	25	6	24
	Amanlema	27	14	51.90
	Elanygodage	26	9	34.62
	Kasowarra	28	0	0
	Deneba	28	0	0
	Ketaberenda	26	3	11.52
	Aleltu	27	1	3.700
Arsi		241	69	28.63
	Burkitu	27	6	23.08
	Ashebeka	30	14	46.67
	Shalojigesa	25	7	28
	Gendabusa	27	7	25.93
	Tulujebi	26	7	26.92
	Melkajebi	26	12	46.20
	Gunguma	30	5	16.67
	Munesa	30	9	30
	Guridangago	28	2	7.140
Total		480	103	21.45

Pearson chi^2 (17): 76.0200; Pr: 0.000

46.2% where as no seroprevalence detected in Wajitushebe, Kasowarra and Deneba. There is significant diffirence between the kebele in the seropositivity ($p<0.05$). Seroprevalence increase as one goes from highland to midland. Seroprevalance was found to be high in area near rivers, irrigation canals and water reservoir.

The seroprevalence of AHS is highest in donkey as compared to horse and mules were 24.60, 20.34, 20%, respectively however there is no significant variation of seropositivity was observed between the horses, donkey and mules ($p>0.05$). There was insignificance variation in seroprevalence between the age groups of equines affected by AHS ($p>0.05$). Although the seroprevalence of male in this study was high (23.13%) as compered to female (18.82%), there was no statistically significant difference ($p>0.05$) in seroprevalence as shown in Table 3. The seroprevalence of the AHS in the midland was 31.38% where as in the highland 15.06%. The Variation in the seroprevalence in two agro-ecology (midland and highland) was found to be significant, ($p<0.05$).

Questionnaire survey: The AHS has different name in different study area, in the kebele like Wajitushebe Fasilangeso, Etitusura, is called “killisa” where as in the rest study area known as “garagalcha”and “dibe gammoji”. A total of 90 equine owners were interviewed about African horse sickness but the majority of the owners were found to have no experience of the disease. However, those owners whose livelihoods depend on equine (33%) were able to recognize the disease and the rest nothing. They described the disease affect mostly horses, mules were the second most susceptible and donkeys were seen rarely with the disease.

It was determined that both sexes of equines were equally affected by AHS. The disease was quite frequently seen in lowlands than higher elevations. Most of the respondents were not able to describe the mode of transmission of the disease. Only some of the people (11%) said, that the disease is transmitted by biting fly called “yekolla zinb”, when they are going to brought “boji” feed and for marketing in the lowland area. The disease occurs mostly in the dry season and some times in the rainy season. In the assessment made to know the knowledge base of equine owners about culicoides vectors, most of the respondents were not able to know these vectors and the role they play as well rather, they are totally unaware of them. It was conclusively described that flies are abundant immediately after the rainy season, because the climate is conducive for their multiplication. Equines in the study areas are either stabled but allowing entrance of insects or they are simply tethered without stabling.

Almost all the study areas are located close to permanent water bodies such as Wabeshebelle and Maribo River in Bale zone and in the Arsi zone such as Keter, Welkesa and Keleta River are found. Other water points, water reservoirs and irrigation canals which support insect breeding were common in the study area. The movement pattern of equines from the high elevations to lowlands for bringing of “boji” feed in kolla and for marketing (Delomana) is a predisposing factor for the prevalence of the disease in the study area.

Table 3: Seroprevalence of AHS in different potential risk factors

Factors	Classification	Total tested	Positive	Seroprevalence %	χ^2	p-values
Type of equidea	Horse	349	71	20.34		
	Donkey	126	31	24.60	1.0029	0.606
	Mules	5	1	20		
Age in years	1-4	73	14	19.18	0.2656	0.606
	>4	407	89	21.87		
Sex	Male	286	68	23.13		
	Female	184	35	18.82	1.2569	0.262
Agro-ecology	Highland	292	44	15.06		
	Midland	88	59	31.38	15.1703	0.000
Total		480	103	21.45		

DISCUSSION

The result of this study indicated that seroprevalence of AHS 28.63% in the Arsi, 14.23% in Bale and the overall prevalence of about 21.45% were found. There was significant variation of seroprevalence in between kebele, highland and midland of the study areas; in contrary to this study there was insignificant variation of seropositivity between sex, age and three type of equidae (horse, donkey and mules).

According to the seroprevalence study of AHS conducted by Keith (2005) in different agro- ecological zones of Amhara and Tigray regions seropositivity values of 48% in donkeys and 70.6% in mules were obtained. The seroprevalence finding in donkeys and mules in this study finding is less than that of Keith (2005). In this study seroprevalence finding was 20.34% horse, 24.60% donkeys and 20% of mules were found to be seropositive. Kassa (2006) said that seropositivity increases as one goes from highland to midland and lowland areas. The difference in the seropositivity in the Keith (2005) and in this finding may be due to environmental difference.

The seroprevalence of AHSV antibodies in the three types of equidae (horse, donkeys and mules) was determined insignificant ($p>0.05$). In this study seroprevalence was slightly higher in donkeys (24.60%) than horses (20.34%) followed by mules (20%). According to, Erasmus (1998) and OIE (2004) among equidae, horses are the most susceptible to AHS with a mortality rate of 50-95% followed by mules with mortality around 50%, donkey with mortality rate 5-10%. From this fact, there is 90-95% chance of recovery in donkeys from infection due to AHS unlike horses and mules. In contrary to this, exposed horses and mules often die of AHS viral infection. Field experience conducted by Kassa (2006) during active disease search and serosurvey indicated that, getting cases of seropositive recovered mules and horses after being exposed to natural challenges is rare in the study areas. The higher seroprevalence observed in donkeys in this study is in agreement with what had been said by Kassa (2006).

The seroprevalence of AHS in the Arsi (28.63%) and Bale (14.23%), was found in the present study. The seroprevalence was higher in Arsi than the Bale, this is because of the population of Arsi zone has close relationship with the lowland area as compered to Bale and thus there is movement of equine to lowland for marketing as result there is acquiring of the disease from the lowland area. White (1997) reported that clucoides vector which can transmit the AHSV are greatly abundant from May to November, some parts of Arsi, This is why the seroprevalence of AHS higher in the Arsi than the Bale in this study.

In this study the seroprevalence of AHSV in the two age categories was assessed. In the findings there was no significant variation in seropositivity between the age groups of equidae ($p>0.05$). Seroprevalence of 19.18% in one to four year age (1-4) and 21.87% in greater than four (>4) year age groups was found. This finding is supported by Keith (2005) in that, all foals that have lost their maternal antibody by six month of age would be protected by vaccination. Keith (2005) further described that different age groups of equidae that are above six month of age had equally seroconverted and protected after they were being vaccinated. From this study and Keith's survey results it can be inferred that all age groups of horse, donkeys and mules seem likely to be equally affected by AHSV.

In the present study the seroprevalence of AHSV was determined in the two sex groups of equidae. A seroprevalence of 18.82% in female and 23.13% in male was found. Kassa (2006) explained that there is no significant variation in seropositivity in the two sex groups; rather they are equally affected by the AHS. Thus in this finding it will agree with that of the Kassa (2006) Where the variation was not significant ($p>0.05$) in the seropositivity between sex.

In this study the seroprevalence of AHS was assessed in two different agro ecological zones of the study areas with 31.38% in midland and 15.06% highland. In the findings there was statistically significant difference of seropositivity of AHS was obtained in the two agro ecological zones of the study areas (midland and highland). According to NVI (1983), the distribution of the disease seems to have positive correlation with the ecology of its vectors. Furthermore, the disease is considered to be endemic to the lowlands and midlands of Ethiopia. However, some cases of the disease are known to appear in surveyed highlands of Ethiopia. So that in this finding disease is in agreement with that of NVI where the diseases are found in midland and highland. According to Radostits *et al*. (2007) also suggested that endemic areas are more likely to be in low-lying, warm and marshy regions that create favourable environment for multiplication of culicoides and mechanical vectors. Mellor *et al*. (1998) described that increased use of irrigation, lakes, manure, urine, dung, tree holes, rotten vegetation, stagnant surface water are ideal larval habitats for the multiplication of culicoides. From the study there is difference in the seropositivity within the midland, This is because of the environmental variation in which river, irrigation canal, water reservoir which are surrounding the study area create conducive environment to the multiplication of the vector as

compared to that of highland. The seropositivity of AHS is higher in the Amanlema (51.9%), Ashebeka (46.6%) and Melkajebi (46%) as compared to other study areas. This could be due to the frequency of movement pattern of equine for the purpose of marketing and bringing of "boji" feed for their cattle to lowlands areas.

Kassa (2006) and Keith (2005) explained that, it is environment rather than species or husbandry that is relevant to seroconvertion. Therefore, from the finding of this study and contextual comparison of the present findings with previous survey results of other authors in different parts of Ethiopia, it is likely to infer that AHS exists in all agro ecological zones of the present study areas (midland and highland) even though lowland was not included in the study.

CONCLUSION AND RECOMMENDATIONS

From the study I can conclude that, the seroprevalence of AHSV was 28.63 and 14.23% in Arsi and Bale zone, respectively. From the total sample tested the seroprevalence of 24.60% in donkeys, 20.34% horses and 20% mules was obtained, the overall prevalence with 21.45%. From finding the African horse sickness affects equally almost all age groups and sexes, hence there is no significant variation in the seropositivity among the equine. In this study the higher prevalence was observed in the midland (31.38%) followed by highland (15.06%). The questionnaire survey result indicates that the knowledge base of equine owners about AHS, mode of transmission and clucoides vector was not satisfying. They are unaware of culicoides vectors and mode of transmission of AHS. Generally AHSV exists in both agro ecological zones (midland and highland) and affect three types of equidae (horse, donkey and mule) in the study areas.

Based on the above conclusion following recommendation are forwarded: There should be awareness reaction about AHS and Clucoides vector among the people through an organized extension package to the present study areas. Appropriate equine enclosure system to avoid insect bite by stabling them some hours before sunset and letting them out a few hours after sun rise as Clucoides are nocturnal in nature and are not inclined to enter equine stables and also control of insect using chemical or biological method. All equine greater than 6 month in the study area should be vaccinated with polyvalent vaccine in the study areas. There is a need of deeper understanding of the epidemiology of African horse sickness in the study areas by an integrated approach of serotyping and identification of the clucoides vectors as well as other potential vectors.

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